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14. ABSTRACT
One brain hemisphere from 32 individuals with idiopathic autism, 12 subjects with duplications 15q11.2-q13/autism and 28 control subjects has been embedded in celloidin or polyethylene glycol, cut into serial 200 µm or 50 µm thick serial sections. The preserved material has been used for morphometric studies in Project 2. Application of neuropathological exclusion criteria (comorbidity, pre- and peri-mortem alterations, autolysis) reduced the risk of distortion of morphometric studies and revealed different pattern of developmental changes in autism with unknown and known etiology. The study revealed microcephaly in duplications 15q11.2-q13, heterotopias and dysplastic changes in the hippocampus in 89% of dup 15 cases (10% in idiopathic autism). Cerebral cortical dysplasia was found only in idiopathic autism (50%). The detected microcephaly, topography and severity of developmental abnormalities explained the higher risk of early onset of seizures and sudden death in dup(15). The study of developmental abnormalities in the hypothalamic nuclei and in the serotonergic system in raphé nuclei revealed a severe delay of neuronal growth in structures known to have trophic functions, acting as a potential initiator/enhancer of the developmental delay detected in Project 2.

15. SUBJECT TERMS- Autism neuropathology, chromosome 15 duplication, microcephaly, heterotopia, dysplasia, subependymal nodular dysplasia, hypothalamus, serotonergic system, desynchronized neuronal growth, clinico-pathological correlations

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FINAL REPORT
2013

Program Project Title: Characterization of the pathological and biochemical markers that correlate to the clinical features of autism

Program Project PI: Jerzy Wegiel, Ph.D.; Co-PI: W. Ted Brown, M.D., Ph.D.

SUBPROJECT 1

The neuropathological markers of abnormal brain development and aging in autism

Subproject 1 P.I.: Thomas Wisniewski, M.D.

INTRODUCTION

The study of neuropathological, morphometric and biochemical markers of autism was designed to detect correlations between pathology and clinical manifestations of autism, and to identify developmental alterations that might be targets for treatment.

The project was executed consistent with the original Program Project and Project 1 aims and timetable.

Material : We examined brains of 72 subjects including: 32 brains of autistic people, 12 brains of individuals with autism associated with chromosome 15 duplication (dup15) and 28 brains of control subjects, exceeding the original plan by approximately 30%.

Aims: Project 1 plays a dual role in the Program Project and its function is reflected in the technical and research aims:

1. To preserve tissue from 72 brains, according to a standardized protocol for neuropathological studies (Project 1) and for morphometric studies (Project 2).
2. To implement clinical inclusion and neuropathological exclusion criteria to reduce the risk of distortion of morphometric studies by comorbidity, postmortem tissue changes, and pathology associated with mechanisms leading to death. Cases with signs of comorbidity, premortem and postmortem changes affecting brain structure were excluded from the morphometric studies.
3. To determine the type, topography and severity of developmental changes including defects of neurogenesis, migration and dysplastic changes, and to estimate their contribution to epilepsy and epilepsy related mortality.
4. To establish protocols of tissue preservation for neuropathological and morphometric studies of two regulatory systems: serotonergic system and hypothalamic system with known contribution to behavioral changes in autism and with a crucial role in trophic control of neuronal growth in the entire brain.

Tissue preservation. Tissue preservation included: brain hemisphere fixation, dehydration, embedding, cutting, staining/immunostaining. Two standardized protocols were applied: celloidin and polyethylene glycol (PEG) embedding protocols. The celloidin protocol provides 200-µm-thick sections mainly for brain neuropathology and morphometry. The PEG protocol provides 50-µm-thick sections for neuropathology and immunocytochemistry-based morphometry. Application of both protocols results in preservation of the entire brain hemisphere for a unique power (a) extended protocol of neuropathological evaluation detecting each abnormality more than 1 mm in diameter and (b) complex morphometric study of 36 brain anatomic structures and their cytoarchitectonic subdivisions selected to monitor potential link between structural developmental defects and social and communication deficits, ritualistic behaviors, intellectual deficits, and seizures.

Data acquisition, storage and processing. Tissue processing, distribution and analysis were monitored by a computerized system of brain tissue samples, sections and histological slides trafficking and storage. This Neuropathological Database was linked to the Project 2 Morphometric Database.

BODY

1. Differences Between the Pattern of Developmental Abnormalities in Autism Associated with Duplications 15q11.2-q13 and Idiopathic Autism

Wegiel J, Schanen CN, Cook EH, Sigman M, Brown WT, Kuchna I, Nowicki K, Wegiel J, Imaki H, Ma SY, Marchi E, Wierzba-Bobrowicz T, Chauhan A, Chauhan V, Cohen IL, London E, Flory M, Lach B, Wisniewski T. J Neuropathol Exp Neurol 2012, 71, 382-397.

Approximately 5%–10% of individuals with an ASD have an identifiable genetic etiology corresponding to a known single gene disorder, such as fragile X syndrome, or chromosomal rearrangements, including maternal duplication of 15q11-q13. The symptoms in people with idic(15) markers are correlated with the extent of duplication of the Prader Willi syndrome/Angelman syndrome critical region (PWS/ASCR) (15q11-q13) (Cheng et al 1994, Robinson et al 1993). Larger supernumerary idic(15) chromosomes, are associated with a cluster of clinical features that include autistic behavior, intellectual deficit (ID), seizures, hypotonia, hyperactivity, and irritability (Wisniewski et al 1979). Duplications of chromosome 15q11-q13 account for approximately 0.5%–3% of ASD and may be the most prevalent cytogenetic aberration associated with autism in most studies (Cook et al 1997, Schroer et al 1998, Gillberg et al 1991, Ghaziuddin et al 1993, Baker et al 1994, Bunday et al 1994).

We hypothesized that neuropathology of autism with dup(15) differs from that of idiopathic autism and provides an explanation for the high prevalence of seizures and associated sudden death in dup(15) cohort. The purpose of this study was to detect the difference between the patterns of developmental abnormalities in the brains in autism of unknown etiology (idiopathic autism) and individuals with duplications of chromosome 15q11.2-q13 [dup(15)] and autism, and to identify alterations that may contribute to seizures and sudden death in dup(15) syndrome. Brains of the 9 subjects with dup(15), 10 with idiopathic autism and 7 control subjects were examined. In dup(15) cohort seven subjects (78%) were diagnosed with autism, 7 had seizures (78%), and in 6 cases (67%) sudden unexplained death was reported. Subjects diagnosed with dup15 autism were microcephalic, with the mean brain weight 300g less (1,177 g) than in idiopathic autism (1,477 g; $p<0.001$). Heterotopias in the alveus, CA4 and dentate

gyrus, and dentate gyrus dysplasia were detected in 89% of subjects with dup(15) autism but in only 10% of subjects with idiopathic autism ($p<0.001$). However cerebral cortex dysplasia was detected in 50% of subjects with idiopathic autism and in none of dup(15) autism cases ($p<0.04$). Different spectrum and higher prevalence of developmental neuropathological changes than those seen in idiopathic autism may contribute to high risk of early onset of seizures and sudden death in the dup(15) cohort.

2. Clinicopathological Stratification of Idiopathic Autism and Autism Associated with Duplications 15q11.2-q13

Wegiel J, Schanen NC, Cook EH, Brown WT, Kuchna I, Nowicki K, Wegiel J, Imaki H, Ma SY, London E, Wisniewski T. Chapter in press: The Neuroscience of Autism Spectrum Disorders; Editors: Joseph Buxbaum and Patrick Hof, Elsevier Inc. 2013

Postmortem studies of brains of individuals with idiopathic autism and duplications 15q11.2-q13 autism identify a cluster of neuropathological features differentiating these cohorts. They show a need for both sub-classification of autism according to etiology, clinical presentation, and neuropathology, and a commonality of clinical and neuropathological traits justifying autism diagnosis. The features differentiating these cohorts include: (a) maternal origin dup(15), (b) autism in 78% of subjects, (c) more severe clinical phenotypes, with intellectual deficit (100%), early-onset of severe or intractable seizures in 78% of subjects, and increased to 67% prevalence of sudden unexplained death, (d) high prevalence of microcephaly, with mean brain weight 300g less than in idiopathic autism, (e) several-fold increase in the number of developmental abnormalities, including defects of migration and dysplastic changes, especially numerous in the hippocampal formation, and (f) significant increase of the intraneuronal amyloid load, reflecting enhanced amyloid-β precursor protein processing with α-secretase.

2. Developmental alterations of raphe nuclei in autistic subjects 5-15 years of age – Methods and technical limitations.

Wegiel Jarek, CUNY, Ph.D. Dissertation 2013, pp 1-135

The role of the serotonergic system in autism is supported by more than 500 reports. They reveal a link between serotonergic system alterations and social deficits, repetitive behavior, hyperactivity, anxiety and obsessive compulsive behavior observed in autism:

1. Increased prevalence of hyperserotonemia in the blood in autistic subjects (Schain and Freedman 1961, Cook and Leventhal 1996, McBride et al. 1998, Hranilovic et al. 2007, and Melke et al. 2008).
2. The association between hyperserotonemia and increased risk of recurrence of autism within families (Cook et al. 1990, Piven et al. 1991, Cross et al. 2008).
3. The correlation between blood serotonin level and severity of clinical symptoms (Hérault et al. 1996).
4. The correlation between low level of serotonin precursor (tryptophan) and severity of stereotyped behaviors in autistic subjects (McDougle et al. 1996).
5. Impairment of the serotonergic system in the brain of autistic subjects (Chugani et al. 1997, 1999, Makkonen et al. 2008).
6. Link between brain serotonin deficit and severity of social deficits (Chugani et al. 1999)
7. Link between reduced uptake of tryptophan and severe language deficits (Chandana et al. 2005).

8. Amelioration of obsessive compulsive behavior, anxiety and aggression in some subjects treated with SSRIs (McDougle et al. 1996, de Long et al. 1998, Fatemi et al. 1998, Hollander et al. 2005, Kolevzon et al. 2006).
9. Abnormalities of serotonergic fibers in many target cortical and subcortical structures known to be involved in autism phenotype (Azmitia et al. 2011a).
10. Azmitia (2011b) review reveals links between serotonergic system alterations and epilepsy, immune dysregulation, gastrointestinal and sleep disorders, as well as anxiety, aggression, obsessive compulsive behavior and mood disorders observed in autism.

However, in spite of evidence of altered development of brain serotonergic system and contribution of these alterations to the autism phenotype, the raphé nuclei, which are the source of brain serotonin, have not been examined.

The aim of this stereological and quantitative immunofluorescence-based study of raphé nuclei in autistic subjects 5 to 15 years of age and age matched control subjects was to (a) establish methods of preparation, staining, and analysis of fixed human brainstem samples obtained from brain banks, and (b) characterize the pattern of developmental abnormalities which may contribute to the autistic phenotype.

Routine neuropathological brainstem dissection results in partial or complete loss of raphé nuclei integrity. From 9 autistic and 6 control subjects only four pairs 5 to 15 years of age were qualified for the study of raphé nuclei. Formalin-fixed brainstem was dehydrated and embedded in polyethylene glycol and cut into serial 50-μm-thick sections. They were stained to estimate cell volume, and immunostained and examined by fluorescence microscopy to estimate the amount of tryptophan hydroxylase (TPH) which is a measure of serotonin synthesis level.

3-D reconstruction demonstrated topography and size of raphé nuclei and explained why preservation of raphé nuclei located in the midline required modification of brainstem sampling. Nucleator applied to TPH (+) sections revealed 24% smaller neuronal soma volume in the dorsal raphé nuclei of autistic subjects than in control group. Application of immunofluorescence and ImageJ software (NIH) revealed significant increase in tryptophan hydroxylase (TPH) immunofluorescence in spite of smaller size of raphé neurons.

These data indicate developmental impairment of neuron growth comparable to that observed in cortex and in subcortical structures. Enhanced TPH immunofluorescence in raphé neurons was consistent with enhanced immunoreactivity in serotonergic fibers in several brain regions of autistic subjects (Azmitia et al. 2011ab). Pathology detected in raphé neurons suggests that target brain areas were exposed to altered levels of serotonin, which may modify function of cerebral cortex and subcortical structures and contribute to the autistic phenotype.

KEY RESEARCH ACCOMPLISHMENTS

Project 1 has a significant contribution to three major research strategies of the Program Project, including the study of the contribution of:

1. Qualitative developmental abnormalities to the autistic phenotype.
2. Quantitative developmental abnormalities to the autistic phenotype.
3. Developmental neuronal metabolic alterations to the clinical phenotype of autism

Major accomplishments:

1. Because of DOD grant and Autism Speaks and Autism Tissue Program support of tissue acquisition, we were able to preserve the historically largest collection of unique quality brain tissue samples (72 brain hemispheres) including:
   - 32 brain hemispheres of people with idiopathic autism,
   - 12 brain hemispheres of people with dup15 autism,
   - 28 control brain hemispheres.
2. Brain hemispheres cut into serial sections provided material for several research strategies. Therefore, we were able to expand the spectrum of research targets and hypotheses tested in this Program Project.
3. The neuropathological component (Project #1) provided neuropathological reports and exclusion criteria reducing risk of distortion of research results by comorbidity, pre-, peri- and postmortem changes.
4. The study determined the contribution of qualitative developmental abnormalities to the autistic phenotype in autism with unknown etiology and autism caused by maternal origin dup(15) (Wegiel et al 2010, 2012).
5. We identified both (a) differences between the pattern of developmental abnormalities in autism associated with duplications 15q11.2-q13 and autism of unknown origin, and (b) the core neuropathology present in autism regardless of autism etiology.
6. This project results in detection of the global pattern of focal abnormalities distribution and the link between these alterations and early onset of epilepsy, functional regression and an increased risk of Sudden Unexpected Death in Epilepsy (SUDEP).

REPORTABLE OUTCOMES

Publications

1. Wegiel J, Wisniewski T, Chauhan A, Chauhan V, Kuchna I, Nowicki K, Imaki H, Wegiel J, Ma SY, Wierzba-Bobrowicz T, Cohen IL, London E, Brown WT. Type, topography and sequelae of neuropathological changes shaping clinical phenotype of autism. In: Autism: Oxidative Stress, Inflammation, and Immune Abnormalities. Ed.: Abha Chauhan, Ved Chauhan and W. Ted Brown. Taylor & Francis/CRC Press, Boca Raton, FL, 2010, pp. 1-34.

2. Wegiel J, Kuchna I, Nowicki K, Imaki H, Wegiel J, Marchi E, Ma SY, Chauhan A, Chauhan V, Wierzba Bobrowicz T, de Leon M, Saint Louis LA, Cohen IL, London E, Brown WT, Wisniewski T. The neuropathology of autism: defects of neurogenesis and neuronal migration, and dysplastic changes. Acta Neuropathol, 2010, 119,755-770.

3. Wegiel J, Schanen CN, Cook EH, Sigman M, Brown WT, Kuchna I, Nowicki K, Wegiel J, Imaki H, Ma SY, Marchi E, Wierzba-Bobrowicz T, Chauhan A, Chauhan V, Cohen IL, London E, Flory M, Lach B, Wisniewski T. Differences Between the Pattern of Developmental Abnormalities in Autism Associated with Duplications 15q11.2-q13 and Idiopathic Autism. J Neuropathol Exp Neurol 2012, 71, 382-397.

4. Wegiel J, Frackowiak J, Mazur Kolecka B, Schanen NC, Cook EH, Sigman M, Brown WT, Kuchna I, Wegiel J, Nowicki K, Imaki H, Ma SY, Chauhan A, Chauhan V, Miller DL, Mehta PD, Cohen IL, London E, Reisberg B, de Leon MJ, Wisniewski T. Amyloid Abnormal Intracellular Accumulation and Extracellular Aβ Deposition in Idiopathic and Dup15q11.2-q13 Autism Spectrum Disorders. PloS ONE 2012, 7, e35414.

5. Wegiel J, Lightfoot D, Pickett J, Brown WT. New trends in brain tissue banking for autism research. Autism Spectrum News 2012, 4, no3.

6. Wegiel J, Kuchna I, Nowicki K, Imaki H, Wegiel J, Ma SY, Azmitia EC, Banerjee P, Chauhan A, Chauhan W, Cohen IL, London E, Brown WT, Wisniewski T. Contribution of Olivo-floccular Circuitry Developmental Defects to Atypical Gaze in Autism. Brain Res 2013 1512:106-122.

7. Wegiel J, Schanen NC, Cook EH, Brown WT, Kuchna I, Nowicki K, Wegiel J, Imaki H, Ma SY, London E, Wisniewski T. Clinicopathological Stratification of Idiopathic Autism and Autism Associated with Duplications 15q11.2-q13. The Neuroscience of Autism Spectrum Disorders; Editors: Joseph Buxbaum and Patrick Hof, Elsevier Inc. Amsterdam 2013, 347-355.

8. Wegiel J, Morys J, Ma SY, Kuchna I, Nowicki K, Imaki H, Wegiel J, Flory M, Brown WT, Wisniewski T. Delayed development of claustrum in autism. Editors: John Smythies, Lawrence Edelstein, V.S. Ramachandran; Functional Neuroanatomy of the Claustrum. Elsevier In press, 2013

9. Wegiel Jarek. Developmental alterations of raphe nuclei in autistic subjects 5-15 years of age - Methods and technical limitations. CUNY, PhD Dissertation 2013; pp. 1-120

10. Frackowiak J, Mazur-Kolecka B, Kuchna I, Brown WT, Wegiel J. The link between intraneuronal N-truncated amyloid-β peptide and oxidatively modified lipids in indiopathic autism and dup(15q11.2-q13)/autism. Acta Neuropathol, submitted 2013

11. Wegiel J, Flory M, Kuchna I, Nowicki K, Ma SY, Imaki H, Wegiel J, Cohen IL, London E, Brown WT, Wisniewski T. Brain-region-specific alterations of the trajectories of neuronal volume growth throughout the lifespan in autism. Acta Neuropathol, resubmitted 2013

12. Wegiel J, Flory M, Nowicki K, Kuchna I, Ma SM, Imaki H, Wegiel J, Cohen IL, London E, Brown WT, Wisniewski T. No evidence of significant changes in the number of neurons in autistic individuals 37 brain subdivisions. In preparation for publication.

13. Wegiel J, Flory M, Nowicki K, Kuchna I, Ma SM, Imaki H, Wegiel J, Cohen IL, London E, Brown WT, Wisniewski T. Different trajectories of abnormal neuronal growth desynchronize brain development in idiopathic autism and autism associated with dup15. In preparation for publication.

Meetings

2009: Annual International Meeting for Autism Research, Chicago, IL; May 7-9

1. Emerging patterns of neuronal growth desynchronization in autism. Wegiel J, Kuchna I, Nowicki K, Wegiel J, Ma SY, Wisniewski T, Cohen IL, London E, Flory M, Brown WT.
2. Potential contributions of developmental and epilepsy-associated neuropathological changes to sudden, unexpected death in four people with chromosome 15 duplication and autism. Brown WT, Wisniewski T, Cohen I, London E, Flory M, Kuchna I, Nowicki K, Wegiel J, Ma SY, Imaki H, Wegiel J.
3. Delayed development of neurons in networks involved with stereotypic behaviors and reward in autism. Nowicki K, Kuchna I, Wegiel J, Ma SY, Wisniewski T, Cohen IL, London E, Flory M, Brown WT, Wegiel J.
4. Developmental heterochronicity of neuron growth in the memory system of autistic subjects. Kuchna I, Nowicki K, Wegiel J, Ma SY, Wisniewski T, Cohen IL, London E, Flory M, Brown WT, Wegiel J.
5. Contribution of thalamic developmental changes to the autistic phenotype. Ma SY, Kuchna I, Nowicki K, Wegiel J, Wisniewski T, Cohen IL, London E, Flory M, Brown WT, Wegiel J.

2010: Annual International Meeting for Autism Research, Philadelphia, PA; May 20-22

6. Defects of neurogenesis, neuronal migration and dysplastic changes in the brains of autistic subjects. Wegiel J, Kuchna J, Nowicki K, Imaki H, Wegiel J, Marchi E, Ma SY, Chauhan A, Chauhan V, Cohen IL, London E, Brown WT, Wisniewski T.
7. Repetitive and stereotyped behaviors in autism are driven by abnormal development of the striatum but not of the substantia nigra. Nowicki K, Wisniewski T, Kuchna I, Wegiel J, Imaki H, Ma SY, Cohen IL, London E, Flory M, Brown WT, Wegiel J.
8. Neuronal growth delay within the claustrum of autistic subjects. Ma SY, Kuchna I, Nowicki K, Wegiel J, Imaki H, Cohen IL, London E, Flory M, Brown WT, Wisniewski T, Wegiel J.

2011: Annual International Meeting for Autism Research, San Diego, CA, May 12-14

9. Neuropathology of idiopathic autism and autism associated with chromosome 15 duplication. Wegiel J, Kuchna I, Nowicki K, Ma SY, Wegiel J, Frackowiak J, Mazur Kolecka B, Marchi E, Cohen IL, London E, Brown WT, Wisniewski T.
10. The olivo-floccular circuitry developmental defects in autism. Kuchna I, Imaki H, Nowicki K, Ma SY, Wegiel J, Cohen IL, London E, Flory M, Brown WT, Wisniewski T, Wegiel
11. Hypothalamic neurons developmental delay in autistic subjects. Ma SY, Kuchna I,

Nowicki K, Wegiel J, Imaki H, Cohen IL, London E, Flory M, Brown WT, Wisniewski T, Wegiel J.
12. Defects of cholinergic neurons development in autism. Nowicki K, Kuchna I, Ma SY, Wegiel J, Imaki H, Cohen IL, London E, Flory M, Brown WT, Wisniewski T, Wegiel J.
13. Accumulation of amyloid-beta peptide species in four brain structures in children with autism. Frackowial J, Mazur Kolecka B, Izabela K, Nowicki K, Brown WT, Wisniewski T, Wegiel J.

2012: NIH meeting, Dup14/autism Alliance meeting, Cell Development Conference
14. Results of application of new methods of tissue handling, distribution, and sharing for research on autism. Wegiel J, Schanen NC, Cook EH, Brown WT, Kuchna I, Nowicki K, Wegiel J, Imaki H, Ma SY, London E, Wisniewski T. 20th Anniversary of the NICHD Brain and Tissue Bank for Developmental Disorders. Contributions of postmortem tissue to the study of developmental disorders. Bethesda, July 16-17, 2012
15. Clinicopathological stratification of idiopathic autism and autism associated with duplications 15q11.2-q13. Wegiel J, Schanen NC, Cook EH, Brown WT, Kuchna I, Nowicki K, Wegiel J, Imaki H, Ma SY, London E, Wisniewski T. Dup15q Alliance 2012 Scientific Meeting. Boston Children's Hospital, Boston August 9-10, 2012
16. Amyloid-beta and lipid oxidation in the brain cortex in autism. Frackowiak J, Mazur-Kolecka B, Kuchna I, Brown WT, Wegiel J. Cell Development Meeting at Santa Cruz, August 2012.

2013: Annual International Meeting for Autism Research, San Sebastian, Spain, May 3-5
17. Global pattern of delayed and desynchronized neuron growth in the brain of autistic subjects. J. Wegiel, M. Flory, I. Kuchna, K. Nowicki, S.Y. Ma, H. Imaki, J. Wegiel, I.L. Cohen, E. London, T. Wisniewski, W.T. Brown
18. Abnormalities in Raphe Nuclei of Autistic 5 to 15 Year Old Subjects. Jarek Wegiel, Efrain C. Azmitia, Thomas Wisniewski, Probal Banerjee

CONCLUSIONS

Focal dysplasia and heterotopias are a major cause of increased risk of epilepsy and sudden unexpected death in autism, especially in autism associated with dup15

1. Idiopathic autism is associated with focal developmental abnormalities in 92% of cases including:
(a) Multifocal cerebral neocortical dysplasia observed in 8% of subjects;
(b) Multifocal archicortex dysplasia in 15%;
(c) Cerebellar dysplasia in 62%;
(d) Subcortical, periventricular, hippocampal and cerebellar heterotopias reflect abnormal neuronal igration in 31%;
(e) Thickenning of the subependymal cell layer and subependymal nodular dysplasia and an indicative of active neurogenesis in autistic children.

2. Dup(15) autism is associated with enhanced developmental alterations:

(a) Severe microcephaly, with brain weight reduced by 300 g, is one of the most significant signs of global encephalopathy increasing the risk of epilepsy.
(b) 2.8 times more frequent developmental alterations, especially common in the hippocampal formation of autistic subjects with dup15, and presence of up to 11 different types of developmental alterations in the brain of a single subject diagnosed with dup(15) autism are a major contributor to early onset of epilepsy and high risk of SUDEP.
(c) Reduced volume of neurons in a majority of subcortical structures and some cortical regions in the brain of autistic children 4–8 years of age appear to reflect brain immaturity in early childhood contributing to autism and intellectual deficits.
(d) Combination of all of these developmental defects increases risk of death at a very early age (~10 years) in autism associated with dup15.

Neuropathology of serotonergic system in autism.

1. This first study of raphé nuclei of 5 to 15 year old autistic subjects revealed:
   a) Significant interindividual differences of the pattern of morphological changes, most likely reflecting contribution of genetic and pre- and postnatal alterations to postmortem detected changes.
   b) Increase in TPH immunoreactivity per cell body that appears to reflect increased level of serotonin in the interfascicular nucleus.
   c) Reduced volume of neuron soma in DRN consistent with numerous reports published in the past two decades demonstrating small neuron size in cerebral cortex, subcortical structures, and cerebellum in autistic subjects.
2. The study recognized several limitations of research on raphé nuclei of autistic subjects including:
   a) Low number of postmortem brain donations of autistic subjects;
   b) Limited number of pediatric control donations;
   c) Conflict between routine methods of brainstem dissection and the need for preservation of anatomical integrity of brainstem serotonergic system for postmortem studies.
3. Standardization of brainstem preservation, immunostaining and methods of quantitative analysis, especially immunofluorescence-based measurements of key proteins reflecting raphé structure and function, will contribute to detection and characterization of serotonergic system alterations in autism.

References

Azmitia EC, Singh JS, Hou XP, Wegiel J. Dystrophic serotonin axons in postmortem brains from young autism patients. Anat Rec 2011a, 294, 1653-1662

Azmitia EC, Singh JS, Whitaker-Azmitia JM. Increased serotonin axons (immunoreactive to 5-HT transporter) in postmortem brains from young autism donors. Neuropharmacology 2011b, 60, 1347-1354

Baker P, Piven J, Schwartz S, et al. Brief report: Duplication of chromosome 15q11-13 in two individuals with autistic disorder. J Autism Develop Dis 1994;24:529–35

Bundey S, Hardy C, Vickers S, et al. Duplication of the 15q11-13 region in a patient with autism, epilepsy and ataxia. Dev Med Child Neurol 1994;36:736–42

Cheng S, Spinner NB, Zackai EH, et al. Cytogenetic and molecular characterization of inverted duplicated chromosomes 15 from 11 patients. Am J Hum Genet 1994;55:753–9

Cook EH Jr, Lindgren V, Leventhal BL, et al. Autism or atypical autism in maternally but not paternally derived proximal 15q duplication. Am J Hum Genet 1997;60:928–34

Cook EH, Leventhal BL, Heller W, Metz J, Wainwright M, Freedman DX. Autistic children and their first-degree relatives: relationships between serotonin and norepinephrine levels and intelligence. J Neuropsychiatry Clin Neurosci 1990, 2, 268-274

Cook EH, Leventhal BL. The serotonin system in autism. Curr Opin Pediatr 1996, 8: 348-354.

Chandana SR, Behen ME, Juhasz C, Muzik O, Rothermel RD, Mangner TJ, Chakraborty PK, Chugani HT, Chugani DC. Significance of abnormalities in developmental trajectory and asymmetry of cortical serotonin synthesis in autism. Int J Dev Neurosci 2005, 23, 171-182

Chugani DC, Muzik O, Rothermel R, Behen M, Chakraborty P, Mangner T, da Silva EA, Chugani HT. Altered serotonin synthesis in the dentatothalamocortical pathway in autistic boys. Ann Neurol 1997, 42, 666-669

Chugani DC, Muzik O, Behen M, Rothermel R, Janisse JJ, Lee J, Chugani HT. Developmental changes in brain serotonin synthesis capacity in autistic and nonautistic children. Ann. Neurol 1999, 45, 287-295

Cross S, Kim SJ, Weiss LA, et al. Molecular genetics of the platelet serotonin system in first-degree relatives of patients with autism. Neuropsychopharmacology 2008, 33, 353-360

DeLong Robert G, Teague LA, Kamran MM. Effects of fluoxetine treatment in young children with idiopathic autism. Dev Med & Child Neurol. 1998, 40, 551-562

Fatemi SH, Realmuto GM, Khan L, Thuras P. Fluoxetine in treatment of adolescent patients with autism: a longitudinal open trial. J. Autism Dev Disord. 1998, 28, 303-307

Ghaziuddin M, Sheldon S, Venkataraman S, et al. Autism associated with tetrasomy 15: A further report. Eur Child Adoles Psychiatry 1993;2:226–30

Gillberg C, Steffenburg S, Wahlstrom J, et al. Autism associated with marker chromosome. J Am Acad Child Adol Psych 1991;30:489–94

Hérault J, Petit E, Martineau J et al. Serotonin and autism: Biochemical and molecular biology features. Psychiatry Res 1996, 65, 33-43

Hollander E, Phillips A, Chaplin W, et al. A placebo controlled crossover trial of liquid fluoxetine on repetitive behaviors in childhood and adolescent autism. Neuropsychopharmacology 2005, 30, 582-589

Hranilovic D, Bujas-Petkovic Z, Vragovic R, Vuk T, Hock K, Jernej B, Hyperserotonemia in adults with autistic disorder. J Autism Dev Disord 2007, 37, 1934-1940

Kolevzon A, Mathewson KA, Hollander E. Selective serotonin reuptake inhibitors in autism: a review of efficacy and tolerability 2006, 67, 407-414

Makkonen I, Riikonen R, Kokki H, Airaksinen MM. Kuikka JT. Serotonin and dopamine transporter binding in children with autism determined by SPECT. Dev Med Child Neurol 2008, 50, 593-597

McBride PA, Anderson GM, Hertzig ME, Snow ME, Thompson SM, Khait VD et al. Effects of diagnosis, race, and puberty on platelet serotonin levels in autism and mental retardation. J Am Acad Child Adolesc Psychiatry 1998, 37, 767-776

McDougle C, Naylor S, Cohen D, Volkmar F, Heninger G, Price L. A double-blind, placebo controlled study of fluvoxamine in adults with autistic disorder. Arch Gen Psychiatry 1996, 56, 1001-1008

Melke J, Goubran-Botros H, Chaste P, Betancur C, Nygren G, Anckarsäter H, Rastam M, Ståhlberg O, Gillberg IC, Delorme R, Chabane N, Mouren-Simeoni M-C, Fauchereau F, Durand CM, Chevalier F, Drouot X, Collet C, Launay J-M, Leboyer M, Gillberg C, Bourgeron T. Abnormal melatonin synthesis in autism spectrum disorders. Mol Psychiatry 2008, 13, 90-98

Piven J, Chase GA, Landa R, Wzorek M, Gayle J, Cloud D, Folstein S. Psychiatric disorders in the parents of autistic individuals. J Am Acad Child Adolesc Psychiatryt 1991, 30, 471-478

Robinson WP, Wagstaff J, Bernasconi F, et al. Uniparental disomy explains the occurrence of the Angelman or Prader-Willi syndrome in patients with an additional small inv dup(15) chromosome. J Med Genet 1993, 30, 756–760

Schain RJ, Freedman DX. Studies on 5-hydroxyindole metabolism in autistic and other mentally retarded children. J Pediatr 1961, 58, 315-320

Schroer RJ, Phelan MC, Michaelis RC, et al. Autism and maternally derived aberrations of chromosome 15q. Am J Med Genet 1998,76, 327–36

Wisniewski L, Hassold T, Heffelfinger J, Higgins JV. Cytogenetic and clinical studies in five cases of inv dup(15). Hum Genet 1979, 50, 259–70

Wegiel, J, Kuchna, I, Nowicki K., Imaki, H., Wegiel, J., et al. (2010). The neuropathology of autism: Defects of neurogenesis and neuronal migration, and dysplastic changes. Acta Neuropath, 119(6), 755–70.

Wegiel J, Frackowiak J, Mazur Kolecka B, Schanen NC, Cook EH, Sigman M, Brown WT, Kuchna I, Wegiel J, Nowicki K, Imaki H, Ma SY, Chauhan A, Chauhan V, Miller DL, Mehta PD, Cohen IL, London E, Reisberg B, de Leon MJ, Wisniewski T. Amyloid Abnormal Intracellular Accumulation and Extracellular Aβ Deposition in Idiopathic and Dup15q11.2-q13 Autism Spectrum Disorders. PloS ONE 2012, 7, e35414.

APPENDICES

1. Wegiel J, Kuchna I, Nowicki K, Imaki H, Wegiel J, Marchi E, Ma SY, Chauhan A, Chauhan V, Wierzba Bobrowicz T, de Leon M, Saint Louis LA, Cohen IL, London E, Brown WT, Wisniewski T. The neuropathology of autism: defects of neurogenesis and neuronal migration, and dysplastic changes. Acta Neuropathol, 2010, 119,755-770.

2. Wegiel J, Wisniewski T, Chauhan A, Chauhan V, Kuchna I, Nowicki K, Imaki H, Wegiel J, Ma SY, Wierzba-Bobrowicz T, Cohen IL, London E, Brown WT. Type, topography and sequelae of neuropathological changes shaping clinical phenotype of autism. In: Autism: Oxidative Stress, Inflammation, and Immune Abnormalities. Ed.: Abha Chauhan, Ved Chauhan and W. Ted Brown. Taylor & Francis/CRC Press, Boca Raton, FL, 2010, pp. 1-34.

3. Wegiel J, Schanen CN, Cook EH, Sigman M, Brown WT, Kuchna I, Nowicki K, Wegiel J, Imaki H, Ma SY, Marchi E, Wierzba-Bobrowicz T, Chauhan A, Chauhan V, Cohen IL, London E, Flory M, Lach B, Wisniewski T. Differences Between the Pattern of Developmental Abnormalities in Autism Associated with Duplications 15q11.2-q13 and Idiopathic Autism. J Neuropathol Exp Neurol 2012, 71, 382-397.

4. Wegiel J, Schanen NC, Cook EH, Brown WT, Kuchna I, Nowicki K, Wegiel J, Imaki H, Ma SY, London E, Wisniewski T. Clinicopathological Stratification of Idiopathic Autism and Autism Associated with Duplications 15q11.2-q13. In: The Neuroscience of Autism Spectrum Disorders; Editors: Joseph Buxbaum and Patrick Hof, Elsevier Inc. 2013, 347-355

5. Wegiel Jarek. Developmental alterations of raphe nuclei in autistic subjects 5-15 years of age - Methods and technical limitations. CUNY, PhD Dissertation 2013; pp. 1-120